The Ochsner Journal 10:231–235, 2010

Innovative Application of Immunologic Principles in Heart Transplantation

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ABSTRACT

Background: Each year, approximately 2,200 heart transplants are performed in the United States. As our understanding of the immune system grows, new tools are being developed to find compatible organ donors and to help with immune surveillance after transplantation. The purpose of this article is to review 3 of these techniques: the virtual crossmatch, the Cylex ImmuKnow assay, and the AlloMap test.

Methods: Two authors (S.A.M. and J.C.) independently performed a literature search with the PubMed database using the key words *ImmuKnow*, *Allomap*, and *virtual crossmatch* in conjunction with *heart transplantation*. Articles were selected for inclusion if they had a primary focus on the use of virtual crossmatch in heart transplantation, the Cylex ImmuKnow assay, and the AlloMap test. Articles were not excluded on the basis of sample size but were excluded if they did not include heart transplant patients.

Results: The virtual crossmatch is a technique that is being used successfully in heart transplant candidates to predict compatibility of donor organs by comparing the potential recipient's HLA-specific antibodies with the HLA type of the prospective donor. The ImmuKnow assay is a noninvasive blood test that measures the strength of immune activity, allowing clinicians to predict risk of infection and possible rejection in heart transplant patients. The AlloMap test is a noninvasive test that quantifies intracellular mRNA levels in mononuclear cells in peripheral blood samples using real-time polymerase chain reaction; this test has been shown to distinguish the dynamic changes in gene expression that occur in the presence or absence of acute cellular rejection.

Conclusion: As the science of transplant immunology advances, transplant cardiologists are taking advantage of the growing fund of knowledge to help their sensitized transplant candidates increase their chances of finding a compatible donor heart and are using commercially available tests to monitor the immune system and rule out rejection after transplantation.

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Key Words: AlloMap, heart transplantation, ImmunKnow assay, rejection surveillance, transplant immunology, virtual crossmatch

INTRODUCTION

Every year in the United States approximately 2,200 heart transplants are performed. Within the first year after heart transplantation, infection and rejection pose the biggest risks to survival, and transplant coronary artery disease (thought to be a form of chronic rejection) and malignancy become greater threats later.[1] As our knowledge of transplant immunology grows, new tools are being developed to help transplant physicians find compatible donor hearts and monitor activity of the immune system after transplant to rule out rejection. This article discusses 3 of these innovative tools: the virtual crossmatch, the Cylex (Columbia, MD) ImmuKnow (IK) assay, and the AlloMap (XDx, Brisbane, CA) test.

METHODS

Two authors (S.A.M. and J.C.) independently performed a literature search with the PubMed database using the key words *ImmuKnow*, *Allomap*, and *virtual crossmatch* in conjunction with *heart transplantation*. Using these search terms, 16 articles were identified. Studies were selected for inclusion if they had a primary focus on the use of virtual crossmatch in heart transplantation (3), the Cylex IK assay (2), and the AlloMap test (3). Articles were not excluded on the basis of sample size but were excluded if they did not primarily focus on the use of these technologies in heart transplant patients.

RESULTS

The Virtual Crossmatch

The presence of antibodies against human leukocyte antigen (HLA) in the blood has been associated with an increased risk of rejection and graft failure. Patients listed for transplant who have a panel of reactive antibodies greater than 10% are considered sensitized and are one of the most disadvantaged groups of recipients on the national waiting list.[2] Formal prospective serologic crossmatching is typically obtained in any patient with a panel of reactive

antibodies exceeding 10% prior to transplantation.[3] Requirement for a prospective crossmatch at the time of transplant usually eliminates the option of long-distance procurement because of the difficulty of transporting donor blood or lymph nodes to the transplant center prior to organ harvesting[2,4] and the need to minimize cold ischemic time once a donor heart reaches its recipient. In the United States, sensitized patients are transplanted at half the rate of their unsensitized counterparts.[5] Additionally, ventricular assist devices (VADs) are increasingly used as a bridge to transplantation in patients who may not survive long enough to receive a heart. Although less reported with continuous flow devices than with the first-generation pulsatile devices, this expansion in VAD use has been associated with increasing rates of allosensitization in cardiac transplant candidates.[6–8]

Recently introduced methods using solid-phase matrices coated with HLA antigens have been shown to detect and identify HLA-specific antibodies with high sensitivity and specificity.[9] These methods have been used to predict compatibility of donor organs by comparing the potential recipient's HLA-specific antibodies with the HLA type of the prospective donor, an approach called *virtual crossmatch*.[2] Antibodies are detected by the use of beads coated with purified HLA class I or II antigens that are incubated with aliquots of the recipient's serum. If HLA-specific antibodies are present in the serum, they will bind to beads coated with the corresponding HLA antigen.[10] Fluorochrome-labeled antihuman immunoglobulin G antibody is used to detect bound antibodies, and beads are run through a laser detector to determine the HLA specificities based on the pattern of reactivity.[10]

Antibody levels are usually considered significant if the mean fluorescence intensity is greater than 2,000 units above the negative control. Donor HLA typing can then be performed by cytotoxicity (serologic testing) or DNA-polymerase chain reaction (PCR) typing. A virtual crossmatch is considered compatible (negative) when none of the recipient's antibodies are directed against the donor's HLA antigens. It is considered incompatible (positive) when any of the recipient's antibodies are directed against the donor's HLA antigens (with mean fluorescence intensity >2,000 units).

Several studies have underscored the utility of virtual crossmatching in vitro, and more recently in clinical scenarios, for both adult and pediatric heart transplant recipients.[10–12] Zangwill et al[11] reported 100% sensitivity in detecting positive flow cytometry crossmatch results for both B and T cells with in vitro analysis of 14 pediatric transplant patients. The authors also reported 72% specificity in predicting a negative T-cell crossmatch and 86% specificity for B-cell crossmatch. In a separate study, Zangwill et al[12] observed highly concordant rates between virtual and retrospective crossmatch, as well as 100% survival of 9 patients transplanted after being listed with a virtual crossmatch. More recently, Stehlik et al[10] reported a 92% negative (compatible) predictive value and a 79% positive (incompatible) predictive value of virtual crossmatch in 257 T-cell antihuman immunoglobulin complement-dependent cytotoxic crossmatch tests. Similarly, 93% negative predictive value was found in retrospective T-cell complement-dependent cytotoxic crossmatch of 14 transplanted patients listed with a virtual crossmatch.

There is limited experience in thoracic transplantation with use of virtual crossmatching without a prospective serologic crossmatch.[11–14] However, increasing evidence around the utility of virtual crossmatching in sensitized patients has led to its incorporation into pretransplant evaluation algorithms by several centers.[4,12] Development of these algorithms such as the one we propose (Figure), the use of VADs as a bridge to transplantation, and use of newer techniques such as virtual crossmatching ultimately intend to ensure equitable access for potential recipients by improved organ allocation, decreased waiting times, and improved survival during waiting time for a compatible donor, while maintaining optimal long-term graft outcomes after transplantation.

Cylex ImmuKnow

Heart transplant patients receive lifelong drug therapy to prevent rejection; however, the amount of drug measured in the blood does not directly correlate with the dose of drug the patient receives, nor does it correlate with the responsiveness of the patient's immune system.[15] Balancing the risk of infection, malignancy, and drug toxicity from oversuppression of the immune system and the risk of rejection and allograft failure from undersuppression of the immune system is one of the major challenges faced by transplant cardiologists.

In 2002, the US Food and Drug Administration approved the use of the Cylex IK assay to measure the suppression of $CD4^+$ T cells, the major target of immunosuppressive therapy in transplant patients. The assay uses the mitogen phytohemagglutinin to stimulate activation of the T cells in a whole blood sample and measures increases in intracellular adenosine triphosphate, the basic energy source for all cells. The strength of the immune response is then reported in nanograms per milliliter of adenosine triphosphate and categorized as low (<225), moderate (226–524), or strong (>525).[16]

In a meta-analysis of retrospective observational studies at 10 centers, including 1,833 IK assays in 504

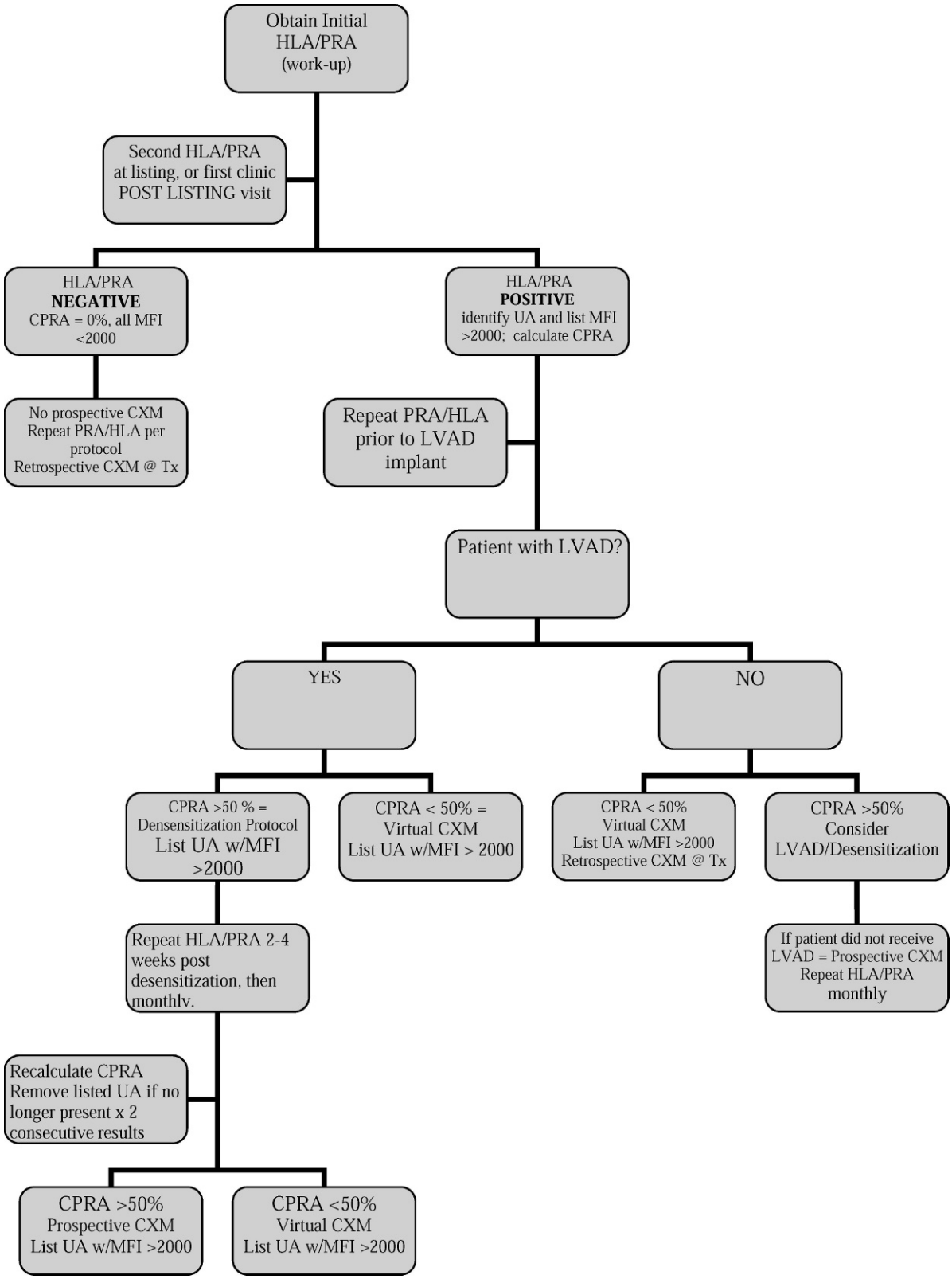

Figure. Pretransplant evaluation algorithm that incorporates virtual crossmatching. HLA: human leukocyte antigen; PRA: panel reactive antibody; CPRA: calculated PRA; MFI; mean fold increase; UA: universal array; CXM: crossmatch; Tx: transplant; LVAD: left ventricular assist device.

transplant patients, the majority of patients with rejection fell into the strong immune response zone, and those with infection had values in the low response zone.[16] Clinically stable patients had moderate responses, and a change of 80 ng/mL indicated a significant change in a patient's immune response. Odds ratios were calculated; a patient with a value of 25 ng/mL was 12 times more likely to develop an infection than a patient with a stronger immune response, and a patient with an IK value of 700 ng/mL was at 30 times greater risk of developing cellular rejection than a patient with a weaker immune response. When the immune responses were compared with the clinical end points of infection or rejection, patients with an IK value between 130 and 450 ng/mL were at minimal risk for infection or rejection, providing a target range for clinicians. However, only 86 of these transplants were cardiac, with only 3 biopsy-proven rejections and 2 clinically diagnosed infections.

More recently, Kobashigawa et al[17] reported their experience with 864 IK assays in 296 heart transplant patients between 2005 and 2008. The assay was performed 2 weeks to 10 years after transplantation and correlated with episodes of infection or rejection that occurred within 1 month of the assay. There were 38 episodes of infection and 8 episodes of rejection. During an episode of infection, the average IK value was significantly lower than the steady-state value (187 vs 280 ng/mL, $P < .001$). The average IK value during an episode of rejection did not significantly vary from steady state (327 vs 280 ng/mL, $P = .35$). An interesting observation in the study was that 3 of the 8 episodes of rejection were antibody-mediated and involved hemodynamic compromise with an IK value that was significantly higher than steady state (491 vs 280 ng/mL, $P < .001$), but further studies are needed, given the small number of rejection episodes.

In conclusion, the IK assay is a noninvasive test that measures the strength of immune activity, allowing clinicians to predict risk of infection and possibly rejection in heart transplant patients. However, the small number of rejection episodes signifies that further studies are needed to conclusively correlate a high IK value with an increased risk of rejection.

AlloMap

The incidence of acute cellular rejection is highest within the first year after transplant (approximately 30%–40%) and lower thereafter.[18] The ''gold standard'' to monitor for acute cellular rejection is endomyocardial biopsy; however, this procedure is invasive, expensive, subject to sampling error and interobserver variability, and associated with rare but potentially life-threatening complications including arrhythmia and ventricular perforation.

The AlloMap test is a commercially available noninvasive test that quantifies intracellular mRNA levels in mononuclear cells in peripheral blood samples using real-time PCR and has been shown to distinguish the dynamic changes in gene expression that occur in the presence or absence of acute cellular rejection.[19] The test yields a score between 0 and 40, with higher scores having a stronger correlation with biopsy-proven rejection.

AlloMap was clinically validated in the Cardiac Allograft Rejection Gene Expression Observational study, in which an 11-gene real-time PCR test prospectively distinguished quiescence from biopsy-proven moderate-severe rejection in 63 asymptomatic patients (*t* test, $P = .0018$).[20] In the study, a score below 30 had a negative predictive value of 99.6% for patients more than 1 year after transplantation, suggesting that the AlloMap might be an alternative to biopsy to rule out rejection in a lower-risk population.

This hypothesis was tested in the Invasive Monitoring Attenuation through Gene Expression (IMAGE) study, in which 602 patients transplanted 6 months to 5 years previously were randomly assigned to be monitored for rejection with either the AlloMap test or endomyocardial biopsy along with clinical and echocardiographic assessment of allograft function.[18] The IMAGE study was a noninferiority study with a composite primary outcome of rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation. At 2 years, the rate of the composite primary outcome was similar in both groups (14.5% AlloMap and 15.3% biopsy; hazard ratio, 1.04; 95% confidence limit: 0.67 to 1.68). Two-year death rates were also similar between AlloMap and biopsy (6.3% vs 5.5%, respectively; $P = .82$) and patients in the AlloMap group had significantly fewer biopsies (0.5 vs 3.0 per person-year, $P = .001$).

Several factors have been found to influence AlloMap score, including time posttransplant, corticosteroid use, and cytomegalovirus. Yamani et al[21] proposed that coronary artery vasculopathy (CAV) would also affect the AlloMap scores, and they evaluated their hypothesis in 69 heart transplant patients with a mean time of 35 months after transplantation. The AlloMap scores of 20 patients with angiographic evidence of CAV were retrospectively compared with 49 patients without CAV. Samples were taken on the same day as scheduled biopsies, and patients with moderate-severe rejection on biopsy were excluded. At baseline, the CAV group had longer mean follow-up (48.7 vs 28.8 months, $P <$

.01), lower ejection fraction (51% vs 60%, $P < .01$), and increased use of sirolimus (40% vs 16%, $P = .034$). Using a logistic regression model and bagging bootstrap approach to account for the time discrepancy and confounders, the investigators found that patients with CAV had higher AlloMap scores than patients without CAV (32.2 ± 3.9 vs 26.1 ± 6.5, $P < .001$). Prospective studies are needed to determine if AlloMap can predict patients who are at high risk for CAV.

CONCLUSION

As the science of transplant immunology advances, transplant cardiologists are taking advantage of the growing fund of knowledge to help their sensitized transplant candidates increase their chances of finding a compatible donor heart and are using commercially available tests to monitor the immune system and rule out rejection after transplantation. Large, randomized prospective trials are needed before these practices can be universally applied as standard of care.

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